REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

The rejection of claims 1 and 15 under 35 U.S.C. § 112 (1st para.) for failure to satisfy the written descriptive requirement is respectfully traversed in view of the above amendments.

The rejection of claims 1 and 15 under 35 U.S.C 103(a) for obviousness over Shoup et al., "Cyclooxygenase-2 Inhibitor NS-398 Improves Survival and Restores Leukocyte Counts in Burn Infection," *J. Trauma* 45(12):215-220 (1998) ("Shoup") in view of U.S. Patent No. 6,552,055 to Spiegelman et al. ("Spiegelman") is respectfully traversed.

Shoup tests the *in vivo* efficacy of the COX-2 inhibitor NS-398 by subjecting mice to a 15% dorsal scald burn plus 1,000 colony-forming units of topical *Pseudomonas aeruginosa* and then administering the COX-2 inhibitor 4-6 hours after the subject is burned and infected (Shoup, pg. 215, abstract; pg. 217, left column, 5th para.). Thus, the subject already has burn sepsis at the point the COX-2 inhibitor is administered (Shoup, pg. 216, left column, 1st and 2nd para.). Shoup does not teach using a selective COX-2 inhibitor in a method for prophylaxis of a patient at risk for systemic inflammatory response syndrome and complications thereof.

Spiegelman teaches inhibiting proliferation of peroxisome proliferator-activated receptor (PPAR)-γ-responsive hyperproliferative cells. This involves ectopically contacting such cells with a PPAR-γ agonist in amounts effective to induce terminal differentiation of the cells. Spiegelman teaches that the PPAR-γ agonist can be used as both a treatment or prophylactic (Spielgelman, column 2, 2nd para.).

It is the position of the U.S. Patent and Trademark Office ("PTO") that it would have been obvious to one of ordinary skill in the pharmaceutical field that if a drug can be used to treat a disease, as in Shoup, it is likewise equally useful for prophylaxis of subjects at risk to suffer from that disease, as described in Spielgelman. Applicants respectfully disagree and submit that the opposite is, indeed, often true.

High doses of antibiotics are commonly used to treat and cure Lyme Disease. However, it is not generally a sound medical practice to administer high doses of antibiotics prophylactically before one is even diagnosed with the disease. Chemotherapy is often used

to treat cancer. However, it would be medically unsound to use chemotherapy prophylactically before cancer has even been detected.

In the outstanding office action, the PTO has acknowledged that Shoup does not teach using a selective COX-2 inhibitor prophylactically on patients at risk for systemic inflammatory response. Combining Spiegelman with Shoup does not remedy this deficiency. Spiegelman teaches the use of PPAR-γ agonists for prevention and treatment of aberrant cell growth. In certain medical situations, like aberrant cell growth, prophylactic and treatment methods may employ the same drugs or medications. However, the PTO has not pointed to any similarities between the PPAR-γ agonists of Spiegelman and the COX-2 inhibitors of Shoup, nor any similarities between the conditions they treat, which would suggest the application of Shoup's methods to prophylaxis. Indeed, the disparate cytokine profiles present in injured animals and injured animals with sepsis would teach away from applying Shoup's treatment prophylactically.

When trauma and/or infection occurs, a body's response includes the up-regulation of Th2-type cytokines and down regulation of Th1-type cytokines, both indicators of systemic inflammatory responses. See Mack et al., "Candida Infection Following Severe Trauma Exacerbates Th2 Cytokines and Increases Mortality", *J. Surg. Res.* 69:399-407 (1997)(Attached hereto as Exhibit A)("Mack"). Employing a mouse model of injury followed by *Candida albican* (CA) sepsis challenge, which induces a systemic inflammatory response, Mack showed a significant increase of Th2 cytokines (IL-4 and IL-6) in these mice over the amount of Th2 cytokines in mice with injury alone (Mack, pg. 402, Figure 3). In addition, Mack showed a significant decrease of the Th1 cytokine IL-2 in injured mice with sepsis when compared with cytokine levels in mice with injury alone (Mack, pg. 401, Figure 2). In view of the different cytokine profiles between mice with injury alone and with injury and infection, one of ordinary skill in the art would not have regarded a regime for treatment of patients that have been injured and infected as being useful in preventing sepsis where only injury has occurred.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: June 23, 2005

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Candida Infection Following Severe Trauma Exacerbates Th2 Cytokines and Increases Mortality[1]

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Presented at the Annual Meeting of the Association for Academic Surgery, Chicago, Illinois, November 13–16, 1996

Following trauma, there is an increase of Th2 cytokines (IL-4, IL-6, and IL-10) and a decrease in Th1 cytokines (IFN-γ and IL-2) that may account for impaired cellular immunity. However, the functional significance of a dominant Th2 pattern to the host remains unclear. The aim of this study was to evaluate whether *Candida albicans* (CA) sepsis in the setting of a Th2 response to trauma leads to increased mortality and to examine the mediators involved. Female BALB/c mice were randomized (12 per group) to receive no injury (C); trauma, consisting of a combined femur fracture and 40% total blood loss (T); no injury plus CA infection (C + CA); and CA infection 1 week following trauma (T + CA). Survival was then followed for 3 weeks. In a separate study, mice were treated as above (5 per group) and sacrificed. Harvested splenocytes were evaluated for concanavalin A-stimulated cytokine production and liver and kidney homogenates were plated to evaluate CA growth per organ and examined histologically. *Candida* infection at 1 week following trauma resulted in significantly increased mortality compared to infected controls. Furthermore, the Th2 dominant cytokine pattern was significantly augmented in the presence of CA infection in both C + CA and T + CA groups. Additional analysis showed significant growth of CA in liver and kidney homogenates from T + CA compared to C + CA mice. These results suggest that injured and infected mice demonstrate augmentation of Th2 dominant responses above that of injury or infection alone, as well as a decreased ability to clear *Candida* which may partially explain the increase in mortality observed. Therapies designed to neutralize Th2 cytokines or augment Th1 cytokines may prove beneficial in the setting of sepsis following trauma. © 1997 Academic Press

INTRODUCTION

Trauma has been demonstrated to increase susceptibility to infectious complications [1]. In fact, infections are the main cause of morbidity and mortality after traumatic injury, accounting for as many as 75% of late deaths [2]. The development of infections after trauma is attributable to loss of barrier functions, tissue destruction, and ischemia, foreign body introduction, and inoculation with microorganisms. Although the host immune mechanisms underlying the increased susceptibility to infections remain unclear, studies have demonstrated a correlation between various injuries including hemorrhage [3–5], burn [6, 7], and trauma [5, 8, 9], with subsequent impairments in the cellular immune response. These impairments have been associated with suppressed T-cell proliferation, decreased CD4/CD8 ratios, and altered production of cytokines [6, 9–11]. The nature of the cytokine profile and release in the local environment reflects ongoing T helper (Th) activities and determines Th1- or Th2-type cytokine responses.

Th1-type cytokines (such as IFN-γ, IL-2, and TNF-β) are known to upregulate cellular immune responses and provide help to B cells for production of opsonizing antibodies, responses which predominate during viral, bacterial, and protozoal infections. Th2-type cytokines (such as IL-4, IL-5, IL-6, and IL-10) downregulate cellular immunity and augment production of nonopsonizing antibodies including IgG1 and IgE, responses involved in allergic reactions and in the elimination of helminthic infections [12–14]. Previous studies determined that certain pathologic states predispose hosts to decreased Th1-type cytokines and inappropriately elevated Th2-type cytokine responses, resulting in defective cellular immunity [10, 11].

Our lab has previously demonstrated in a model of femur fracture and hemorrhage that by 7 days following injury, a strong Th2 dominant pattern of cytokines develops in stimulated splenocytes from BALB/c mice [9]. We hypothesized that this Th2 dominant response may be partially responsible for the host's decreased ability to fight infection. We therefore sought to examine whether mice infected with *Candida albicans* at 7 days following injury, a time at which Th2 cytokines predominate, would display decreased resistance to infection. We then evaluated the cytokine patterns established acutely following *Candidemia* and examined the

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0022-4804/97 $25.00

presence of *Candida* burden at early and late time points following infection by culture growth from kidney and liver homogenates.

METHODS

Animals. Inbred, 6- to 8-week-old female BALB/c mice (body weight 19–22 g) were purchased from Charles River Laboratories (Wilmington, MA). Animals were acclimatized for at least 4 days in the animal facility prior to experiments and allowed food and water *ad libitum*. The animal facility has been approved by the American Association for Accreditation of Laboratory Animal Care, and all animal protocols were approved by the Institutional Animal Care and Use Committee.

Experimental protocol. Mice were randomized to receive either anesthesia alone (control) or anesthesia and a femur fracture plus 40% total blood volume hemorrhage (trauma). Animals were anesthetized with inhaled methoxyflurane (Pitman-Moore, Inc., Mundelein, IL). The right femur of each experimental mouse was fractured by making a 0.5-cm incision on the ventral surface of the mouse in the inguinal region. The intermuscular plane was dissected to expose the proximal one-third of the femur, which was then fractured with sterile scissors. The wound was closed in a single layer with 3.0 Dexon after hemostasis was assured. The 40% total blood volume hemorrhage was determined by the following formula: body weight of the mouse (g) × 75 ml/1000 mg × 0.40 = volume of blood (40%) in milliliters. Bleeding was done via a standard retroorbital approach using a fire-polished, heparinized microcapillary tube. There was an approximate 10% mortality of traumatized mice observed within 30 min of the procedure. All other control and traumatized animals survived until 7 days. At this time, animals from the control and trauma groups were randomized to receive either a 0.1-ml tail vein injection of sterile Hank's balanced salt solution (HBSS) or a 0.1-ml solution containing a total of 750,000 *C. albicans* organisms suspended in HBSS (Gibco).

Animals used in the survival study (n = 12 per group) were followed over a 3-week period before sacrifice with CO_2 asphyxiation. In a separate experiment, animals were treated as above with five mice per group and sacrificed by CO_2 asphyxiation at 4 days after infection with *Candida* via tail vein injection.

For each experiment, fresh *C. albicans* (ATCC 64544) was prepared from a stock solution by incubation for 6 hr in Sabaroud's agar. CA were harvested during the exponential growth phase, centrifuged and washed with HBSS, and suspended in HBSS at a final concentration of 7.5 million CA per milliliter.

Spleens were then harvested from sacrificed mice at 4 days following infection to determine cytokine levels. Livers and kidneys were harvested at 4 days and 2 weeks following CA infection to evaluate CA growth per organ.

Splenocyte preparation. Spleens were collected aseptically and placed in separate petri dishes containing cold (4°C) HBSS without calcium and magnesium (Gibco BRL, Life Technologies, Inc., Grand Island, NY). Spleens were transferred to an antibiotic/antimycotic solution (Gibco BRL, Life Technologies, Inc.) for 1 min and splenocytes were isolated by gentle mechanical disruption of the spleen with filtration through a nylon mesh (Nytex, Tetko, Elmsford, NY) to obtain a single cell suspension. The suspension was centrifuged for 7 min at 500*g* at 4°C. Following resuspension, erythrocytes were lysed with ammonium chloride solution for 4 min and the lysate was centrifuged as described before. The remaining pellet was resuspended in complete RPMI 1640 (Gibco BRL, Life Technologies) containing 2 m*M* L-glutamine, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, and 5% heat-inactivated fetal calf serum (Gibco BRL, Life Technologies, Inc.), counted and diluted to yield a final cell concentration of 5×10^6 cells per milliliter, and plated in 24- and 96-well plates in volumes of 1 and 0.2 ml, respectively. Cells were stimulated in the presence or absence of 2.5 μg/ml Con A (Boehringer-Mannheim), determined as an optimal concentration from a preliminary dose–response study. Splenocytes were then cultured at 37°C in 5% CO_2 incubators and supernatants were harvested at 24 and 72 hr and frozen at −70°C for batch ELISA processing to determine maximal cytokine production.

Cytokine determinations. The capacity of the total splenocyte culture to produce IFN-γ, IL-2, IL-4, and IL-6 was assessed using specific ELISA (all reagents from Pharminogen) according to the manufacturer's protocol. Ninety-six-well ELISA plates were coated with 50 μl/well of purified anti-cytokine (2 μg/ml) capture monoclonal antibody diluted in coating buffer (0.1 M $NaHCO_3$, pH 8.2) and allowed to incubate overnight at 4°C. Plates were washed twice with wash buffer [phosphate-buffered saline/Tween 20 (0.05%)] and blocked with 100 μl/well of blocking buffer (phosphate-buffered saline/10% fetal bovine serum) for 2 hr at room temperature. Plates were washed twice with wash buffer, standards (recombinant cytokines), and samples added and allowed to incubate overnight at 4°C. Wells were washed four times with wash buffer, after which 100 μl/well of biotinylated anti-cytokine (1 μg/ml) detecting monoclonal antibody diluted in blocking buffer was added for 1 hr. The plates were then washed six times with washing buffer and 100 μl/well of avidin–peroxidase solution (1 mg/ml solution diluted 1:400 in blocking buffer) was added for 45 min and allowed to incubate at room temperature. Wells were washed eight times with wash buffer and then 100 μl/well of 2,2'-azino-di[3-ethyl-benzthiazoline sulfonate (6)] peroxidase substrate (ABTS) (Kirkgaard and Perry Laboratories, Gaithersburg, MD) was added and the reaction was allowed to develop at room temperature for 10–60 min. Absorbance at 410 nm was read with a microplate reader. Unknown values were determined from the standard curves generated using a four-parameter curve fit.

Candida culture. After aseptically harvesting livers and kidneys from mice, organs were weighed and suspended in sterile phosphate-buffered saline (PBS) at 1 mg of wet tissue per milliliter of PBS. These samples were then homogenized into solution with HBSS and 10 μl of this solution was plated onto Sabouraud–agarose plates and presence of *Candida* growth was observed over a 5-day period.

Statistical analysis. Survival data are reported as percentage survivors (n = 12 per group) and evaluated by χ^2 test comparing endpoint survival of traumatized-infected (T + CA) versus uninjured infected (C + CA) animals. All cytokine results are reported as means ± SEM of five mice per group. Student's t test was used to determine the significance of the differences between means of C + CA versus uninjured C and of T + CA versus traumatized T groups. Culture data were reported as percentage positive for *Candida* growth (n = 5 per group). Data were evaluated by Fischer's exact test comparing T + CA versus C + CA groups. For all statistical tests, a P value of < 0.05 was considered significant.

RESULTS

Survival

The survival of mice after combination injury and infection with CA is shown in Fig. 1. Whereas control and traumatized mice exhibited 100% survival over the 3-week period, mice infected with 750,000 CA started to die at 11 days following infection and died at a steady rate reaching 70% survival by 24 days. In contrast, although T + CA mice started to die at a similar time (Day 10), by 24 days only 25% of mice had survived ($P < 0.05$ for T + CA versus C + CA comparing endpoint mortality).

Total Splenocyte Cytokine Patterns

IFN-γ levels expressed by total splenocytes are represented in Fig. 2a and show that 11 days following injury (4 days following CA infection), splenocytes from C + CA mice produced levels of IFN-γ (20,484 ± 2581 pg/ml) that were significantly augmented compared to C mice (9655 ± 1610 pg/ml) and were even somewhat

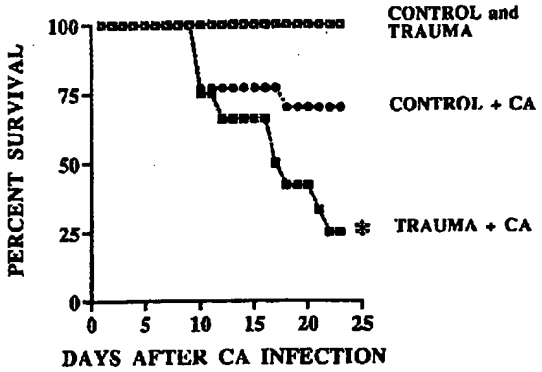


FIG. 1. Survival of mice randomized (12 per group) to receive no injury (C); trauma, consisting of a combined femur fracture and 40% total blood loss (T); no injury plus CA infection (C + CA); and CA infection 1 week following trauma (T + CA). Survival was followed for 1 week following *Candida* infection. *$P < 0.05$ by χ^2 test (T + CA vs C + CA).

higher than levels of IFN-γ produced by splenocytes from T and T + CA groups (17,179 ± 2340 pg/ml and 16,954 ± 1221 pg/ml, respectively). Additionally, we found a significant difference between IFN-γ production of C vs T groups (9655 ± 1610 pg/ml vs 17,179 ± 2340 pg/ml).

IL-2 levels produced by total splenocytes are represented in Fig. 2b and show that IL-2 levels expressed in the T + CA group are significantly decreased compared to the T group (736 ± 45 pg/ml vs 1099 ± 71 pg/ml) ($P < 0.05$ T + CA vs T). Although IL-2 levels are not significantly different in splenocytes from C versus C + CA groups (1158 ± 222 pg/ml vs 749 ± 58 pg/ml), there is a numerical decrease in IL-2 production from splenocytes of infected mice. It is of note that the C + CA and T + CA groups showed similar levels of decreased IL-2 production (749 ± 58 pg/ml vs 736 ± 45 pg/ml).

IL-4 levels are represented in Fig. 3a and show that mice from the C + CA group expressed significantly more IL-4 than splenocytes from the C group alone (193 ± 39 pg/ml versus 65 ± 10 pg/ml) and in the T + CA group, stimulated splenocytes produced significantly more IL-4 than those from the T group (252 ± 62 pg/ml vs 131 ± 22 pg/ml). Additionally, the C + CA and T groups produced levels of IL-4 that were not significantly different from each other. However, the addition of CA in the T + CA group resulted in significantly more IL-4 production compared to the C + CA or T group. A significant difference in the C and T groups was also observed with IL-4 production of 65 ± 10 pg/ml versus 131 ± 22 pg/ml.

IL-6 production by stimulated splenocytes paralleled IL-4 production. Splenocytes from the C + CA group produced significantly more IL-6 than those from the C group (1141 ± 21 pg/ml vs 651 ± 94 pg/ml). Splenocytes from the T + CA group also produced significantly more IL-6 than those from the T group (1291 ± 144 pg/ml vs 922 ± 77 pg/ml). As with production of IL-4, IL-6 levels of splenocytes from C versus T groups demonstrated significant differences of 651 ± 94 pg/ml versus 922 ± 77 pg/ml.

Liver and Kidney Cultures

Table 1 shows the results of plating homogenates of liver and kidney samples from mice in all four experimental groups at 4 days and 2 weeks following CA infection. At 4 days following CA infection, all liver homogenates of mice in the T + CA group grew CA, whereas only 60% of liver homogenates from C + CA mice grew CA. None of the negative controls (C or T) grew CA. In contrast, by 2 weeks following infection, there was a significant difference between groups for growth in the liver. One hundred percent of T + CA

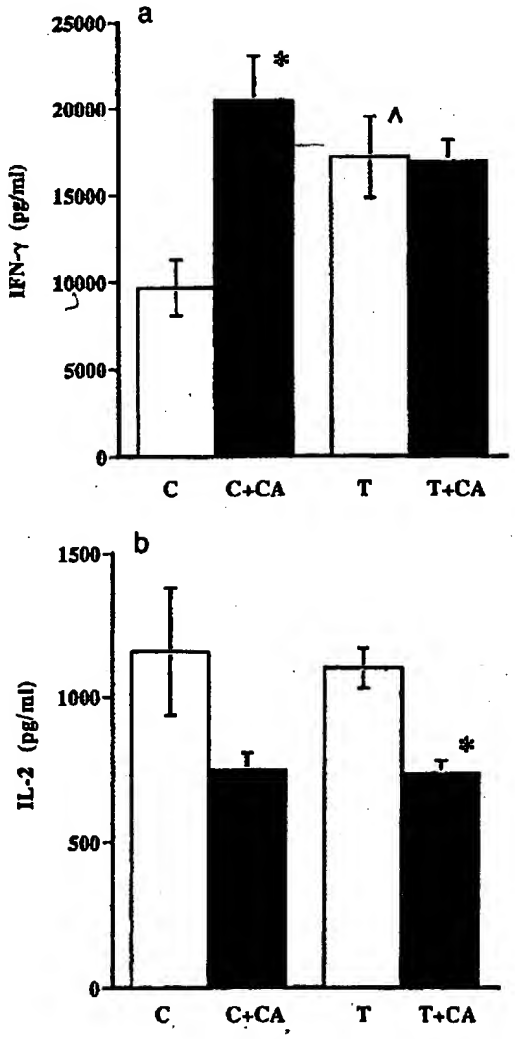


FIG. 2. (a) IFN-γ production by splenocytes from mice randomized to receive no injury (C); no injury plus *Candida* infection (C + CA); trauma, consisting of a combined femur fracture and 40% total blood loss (T); and CA injection 1 week following trauma (T + CA). *$P < 0.05$ (C + CA vs C). ^$P < 0.05$ (C vs T). (b) IL-2 production by splenocytes from mice randomized to receive no injury (C); no injury plus CA infection (C + CA); trauma, consisting of a combined femur fracture and 40% total blood loss (T); and CA infection 1 week following trauma (T + CA). *$P < 0.05$ by Student's t test (T + CA vs T).

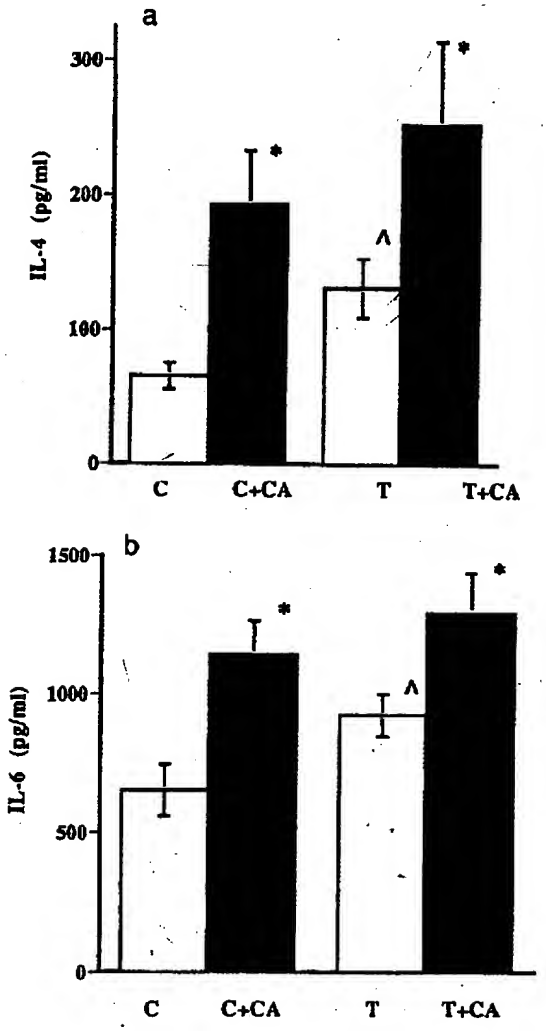


FIG. 3. (a) IL-4 production by splenocytes from mice randomized ($n = 5$ per group) to receive no injury (C); no injury plus CA infection (C + CA); trauma, consisting of a combined femur fracture and 40% total blood loss (T); and CA infection 1 week following trauma (T + CA). *$P < 0.05$ by Student's t test (C + CA vs C and T + CA vs T). ^$P < 0.05$ (C vs T). (b) IL-6 production by splenocytes from mice randomized ($n = 5$ per group) to receive no injury (C); trauma, consisting of a combined femur fracture and 40% total blood loss (T); no injury plus CA infection (C + CA); and CA infection 1 week following trauma (T + CA). *$P < 0.05$ by Student's t test (C + CA vs C and T + CA vs T). ^$P < 0.05$ (C vs T).

cultures grew CA, whereas none of the C + CA cultures showed CA growth.

Kidney homogenates followed a similar trend. At 4 days following CA infection, 80% of T + CA cultures showed CA growth compared to 40% of C + CA cultures. Again, none of the negative controls (C or T) grew CA. This difference was augmented to demonstrate significant differences in CA growth by 2 weeks with 80% CA growth in the T + CA group compared to 0% growth in C + CA cultures.

Histological Analysis

To further examine the results of liver and kidney CA growth, samples of both organs were examined histologically (see Figs. 4a–4d, representing samples at 2 weeks following infection). No remarkable differences were observed in microscopic analysis of the livers by a veterinary pathologist and there was no evidence of fungal growth by silver staining. However, samples at 2 weeks following CA infection show that kidneys from T + CA mice (Fig. 4d) displayed signs of chronic pyelonephritis with areas of inflammation and leukocytic infiltration and collections of leukocytic infiltrates within the collecting ducts. By comparison, kidney samples from C + CA mice (Fig. 4c) demonstrated no such infection, although there was evidence of neoepithelialization of tubular elements consistent with past infection.

DISCUSSION

Following severe injury, it has been well established that the host is at increased risk of developing infectious complications. However, along with emerging changes in the management of patients as well as the use of antibiotics, the source of wound colonization has been changing, especially in patients with trauma and burn injuries [15]. Although colonizers such as *Enterococci, Staphylococcus aureus, Pseudomonas, Bacteroides,* and *Clostridum* remain important sources of infection, *Candida* has been cited as the prominent pathogen among opportunistic yeasts and fungi [15]. In fact, a recent study found that the rate of fungal infections has risen from 2 to 3.8 infections per 1000 discharges with the highest number of nosocomial fungal infections (16.1 per 1000 hospital discharges) found in burn and trauma centers [16]. *C. albicans* was the most frequently isolated fungus, causing 60% of fungal infections, and almost one-third of patients developing fungemia die during hospitalization [16].

Mosmann *et al.* originally developed the concept of Th1 and Th2 cytokine patterns [17], and there has been a plethora of literature and studies lending support to this Th1/Th2 balance associated with the immune response [12, 18–21], although some skeptics remain [22]. According to this classification, Th1 cytokines (IFN-γ, IL-2, and TNF-β) support cellular immunity and initiate the production of opsonizing antibodies such as IgM, IgG2a, and IgG3, responses that aid in eliminating bacterial, viral, and protozoal infections. Th2 cytokines (IL-4, IL-5, IL-6, and IL-10) support non-opsonizing humoral responses with production of IgG1 and IgE, responses involved in mediating allergic reactions and in eliminating helminthic infections.

Many studies in our laboratory and others have established the presence of a cytokine polarization toward a predominant Th2-type cytokine response following various forms of injury [3–5, 7, 23]. Therefore, the aim of the present study was to further evaluate the association between Th2-type cytokines and immune function. Specifically, it was our aim to investigate whether the Th2 cytokine dominance associated with injury (occurring at 7 days after trauma in our model) would correlate with a compromised ability of the im-

TABLE 1

***Candida* Growth from Liver and Kidney Isolates**

Days after CA	*Candida* cultures (% positive for CA growth)							
	Day 4				Day 14			
Exp groups	C	C + CA	T	T + CA	C	C + CA	T	T + CA
Liver	0	60	0	100	0	0	0	100*
Kidney	0	40	0	80	0	0	0	80*

Note. C, control; C + CA, control + *Candida;* T, trauma; T + CA, trauma + *Candida.*
* $P < 0.05$, T + CA vs C + CA by Fischer's exact test, $n = 5$ per group.

mune system to resist an infectious challenge given at the time of this Th2 cytokine response and to examine the mediators involved in this response.

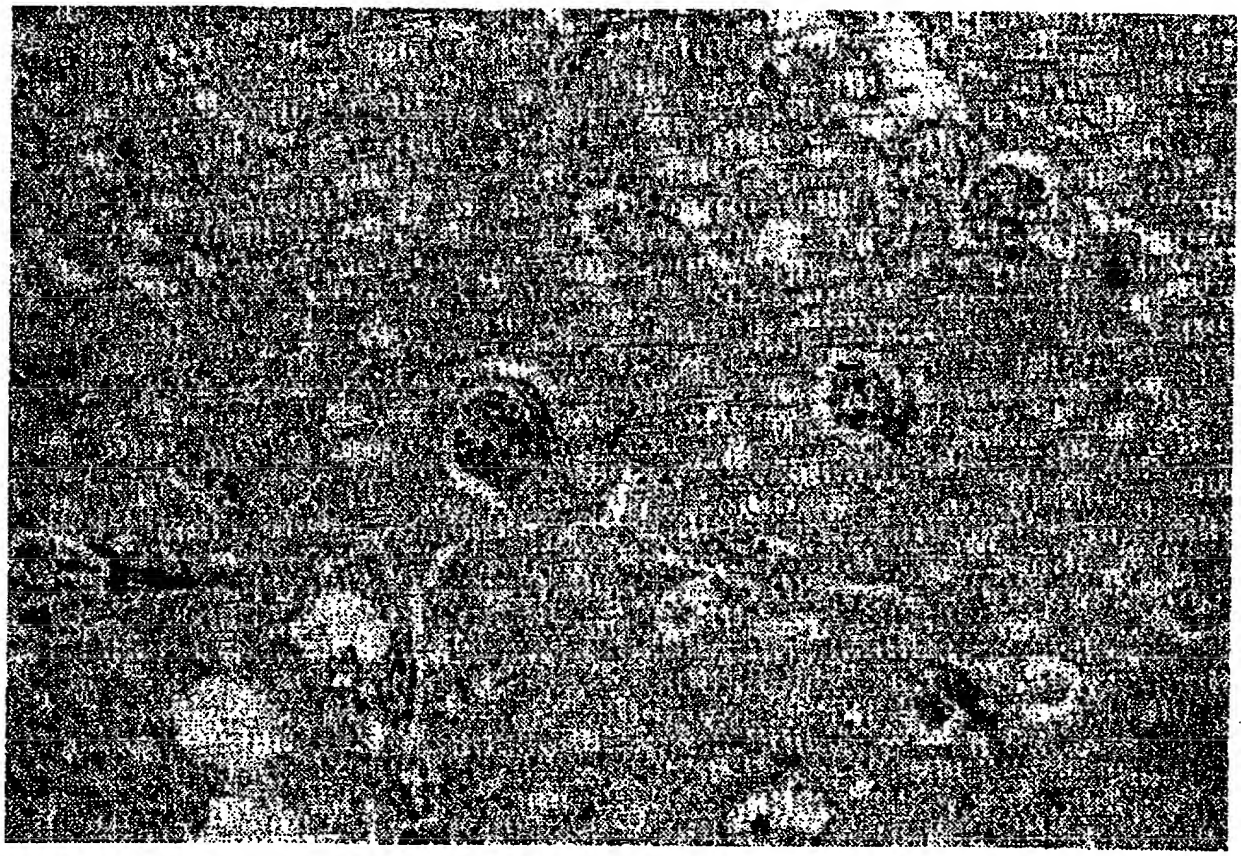
The first phase of the present study supports the hypothesis of altered immune functioning and Th2 cytokines. Mice infected with *Candida* at 1 week after femur fracture and hemorrhage, when their Th2 cytokine patterns have been fully established [9], showed significantly decreased survival at 24 days after infection (25% survival), compared to uninjured mice with infection alone (70% survival). These data show that mice in the T + CA group were less resistant to an infectious challenge with *Candida* than mice in the C + CA group.

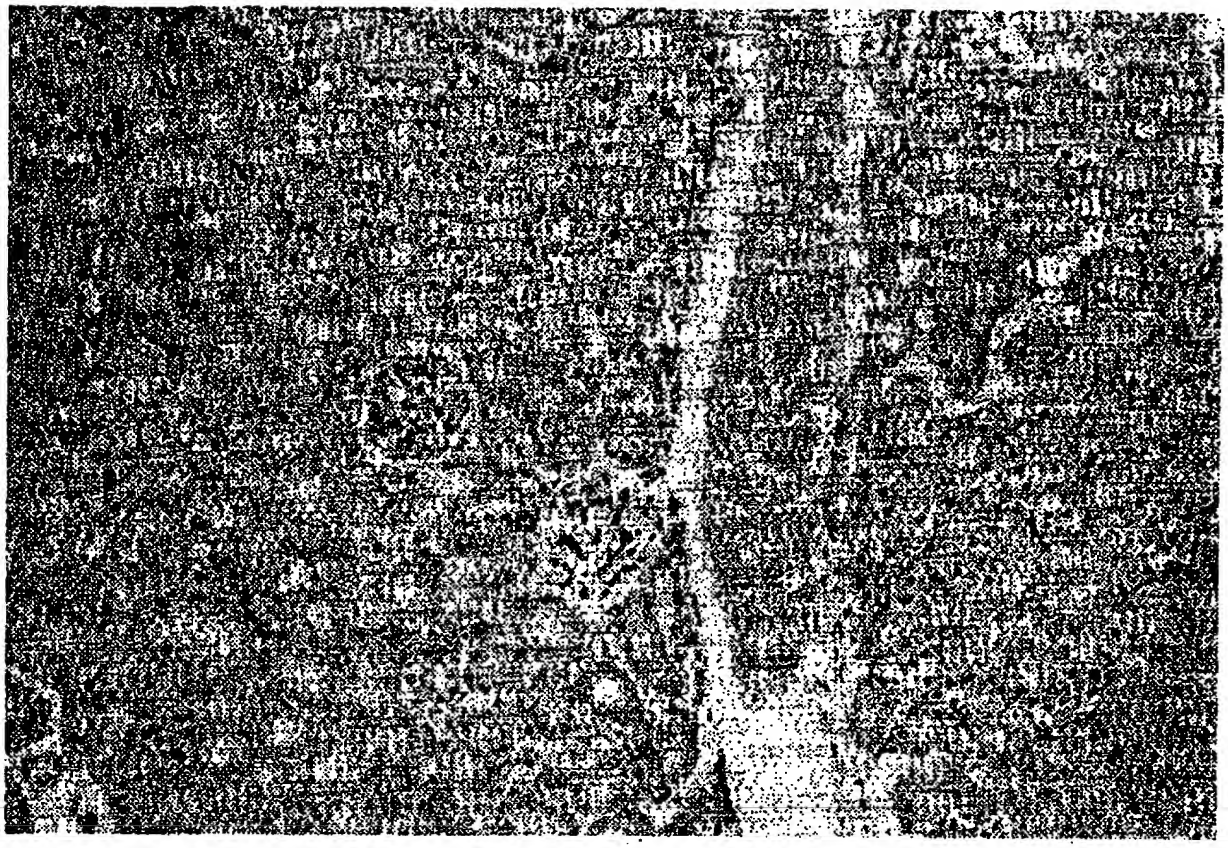
We next investigated some of the mediators associated with this observation, examining the cytokine environment of the T + CA group compared to the C + CA group. This would allow evaluation of how intravenous CA infection, when given to a mouse with an established Th2-type cytokine profile, would effect the Th1/Th2 balance. Several studies have shown that CA infection can induce a nonprotective Th2 dominant response [24, 25], although the relative degree of cytokine response may be strain dependent [26]. However, no studies to our knowledge have examined the effects of CA infection superimposed on traumatic injury in terms of Th cytokine patterns.

In our study, IFN-γ production 11 days following injury (4 days after CA) was the highest in splenocytes from the C + CA group compared to other groups, although increased levels above the control were also observed in T and T + CA groups. Various studies have claimed that fungi stimulate the production of IFN-γ, and the degree may be dependent on mouse strain as well as the fungi burden to the host [26, 27]. In this study, we also found that CA alone induces IFN-γ production. However, at 11 days after injury, there was an increase in IFN-γ production in splenocytes of T mice that was not augmented with the addition of CA infection. The reason for this is currently not known. However, the presence of IFN-γ following infection is believed to predict protective host responses to CA infection that are specific to strains such as BALB/c mice [26]. Such data would suggest that the animals (C + CA) producing the most IFN-γ would be best protected from the infection's effects, as was the case in this study. The other Th1 cytokine, IL-2, shows that infection with CA, either alone or subsequent to injury, causes a decrease in IL-2 production by splenocytes at 4 days after infection. The decrease in IL-2 production was significant in the T + CA group compared to the T group, and IL-2 production was also very low in the C + CA group, suggesting that CA may inhibit or suppress production of IL-2 by a mechanism different than that of trauma alone. For Th1 cytokines, IL-2 clearly cannot contribute to the differing mortalities observed between C + CA and T + CA groups because splenocyte production of IL-2 was similar in the two groups. On the other hand, IFN-γ production increased to a greater extent in splenocytes from C + CA mice versus those from T + CA mice. If one takes this as evidence for a more Th1-type response in C + CA mice, this may help explain their survival advantage over T + CA mice.

The two Th2-type cytokines IL-4 and IL-6 showed similar trends in response to CA infection. Both uninjured and injured mice, after infection with CA, showed significantly increased levels of IL-4 and IL-6, suggesting that the baseline increase in Th2 cytokines caused by injury alone [9] is exacerbated with the addition of CA. Various studies from other laboratories have also found increases in IL-4 and IL-6 production after injury [27]. However, the production of these cytokines in the setting of trauma followed by sepsis has not, to our knowledge, been previously reported. To more completely evaluate the Th2 cytokine profile, future studies should assess the levels of other Th2 cytokines such as IL-5 and IL-10. The augmented levels of IL-4 and IL-6 in T + CA mice could help to explain the increases in mortality, if taken as evidence of a Th2-

FIG. 4. Histological photographs show sections of representative kidney samples from each experimental group. Micrographs from a and b represent C and T groups and do not show appreciable differences in histology. Micrograph c (C + CA), however, shows evidence of neoepithelialization of tubules, although no evidence of active infection. (d) (T + CA) reveals evidence of leukocytic infiltration within interstitial spaces and leukocytic infiltrates within collecting ducts consistent with active pyelonephritis.





type response, since such patterns are associated with decreased resistance to infections and may have rendered the host more susceptible to the effects of *Candida* infection.

Evaluating the growth of CA from homogenates of liver and kidney provided an additional method to evaluate the systemic effects of infection. At Day 4 following infection, some CA growth was observed in both liver and kidney cultures (60 and 40%, respectively, for C + CA mice and 100 and 80%, respectively, for T + CA mice), although these differences were not significant. However, by 2 weeks following infection, none of the C + CA cultures grew CA, whereas liver and kidney cultures of T + CA mice showed continued CA growth (100 and 80%, respectively). This suggests that although both groups displayed signs of acute infection, by 2 weeks, mice in the C + CA group were able to clear infection from the liver and kidney, while CA infection persisted in mice from the T + CA group. This observation is further supported by the histological data which demonstrate that in sections of kidney 2 weeks after infection, the integrity of the C + CA kidney is well preserved, showing no signs of active infection, although there are signs of renal tubular neoepithelialization consistent with previous infection. In contrast, sections from the T + CA kidney showed evidence of leukocytic infiltration within interstitial spaces and leukocytic collections within the collecting ducts, consistent with a diagnosis of active pyelonephritis. These data may also help to explain the increased mortality observed in the T + CA group since 1 week after injury, if it is believed that these mice are immunosuppressed [9], they may be less capable immunologically to clear CA infection than uninjured animals.

Although this study has shown a correlation between increased mortality and augmented production of various Th2-type cytokines, it has not demonstrated a causal relationship. To investigate whether the observed exacerbation of Th2-type cytokines is responsible for decreased immunity and increased mortality, studies could be done to examine the effect of giving uninjured mice Th2-type cytokines such as IL-4, IL-5, IL-6, or IL-10 as well as combinations of these cytokines. Animals might then be infected with CA and followed for survival, comparing them to injured and infected mice. The importance of the production of IFN-γ could also be determined by administering anti-IFN-γ to injured and infected mice and comparing them with a T + CA group.

In summary, it appears that trauma is associated with Th2-type cytokine responses that are further elevated with the addition of CA given in the setting of a Th2 response. The Th2-type exacerbation in the T + CA group compared to trauma alone appears to occur by independent, but additive mechanisms in regard to CA and trauma. The production of IFN-γ, however, may occur by a similar mechanism in regard to stimulation with CA and trauma since the addition of trauma and CA did not increase production of the cytokine to levels higher than trauma alone. Additionally, we found that this exacerbated Th2-type cytokine environment correlates with decreased survival in injured and septic mice compared to septic animals alone. Further studies remain to more accurately determine the mechanism behind this detrimental cytokine response. With an understanding of the mechanisms triggering such cytokine patterns, it may be possible to more clearly investigate therapies to improve the outcome from fungal sepsis.

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